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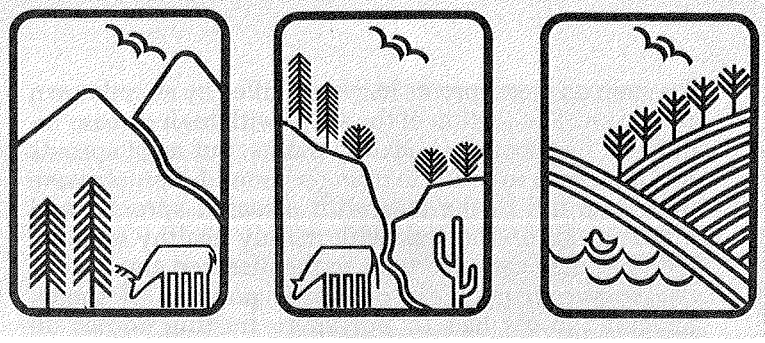
Mountains Southwest Plains



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Fungi Associated with Sprout Mortality in Aspen Clearcuts in Colorado and Arizona

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Four aspen stands with greater than 90% sprout mortality in Colorado and one in Arizona were evaluated for fungal pathogens. These 4- to 5-year-old sprouts had rapidly expanding cankers moving from the terminal to the ground line. Roots of sampled sprouts showed no damage and root diseases were not found. Fruiting structures of *Cytospora chrysosperma* were consistently found on dying stems, while those of *Dothiora polyspora* were occasionally found. The same organisms were isolated from the margins of advancing cankers. Pathogenicity tests using greenhouse-grown aspen trees indicated *C. chrysosperma* caused rapidly expanding cankers similar to those observed on the aspen sprouts, but *D. polyspora* did not cause cankers.

Keywords: *Populus tremuloides*, cytospora canker, *Dothiora*, aspen management

Introduction

Aspen (*Populus tremuloides* Michx.) is the most widely distributed native North American tree species (Little 1971). Colorado and Utah contain most of the aspen in the West where aspen acreage comprises more than 25% of all commercial forests. In Colorado, aspen forests comprise almost 3 million acres and are most prominent west of the Front Range and Sangre de Cristo Mountains (DeByle and Winokur 1985).

Water management, wildlife habitat and food source, timber production, recreation, and scenic beauty are important uses of aspen in the Rocky Mountain region (DeByle and Winokur 1985). A diversity of ages and size classes is required to provide for this mix of demands on the aspen resources. Clear felling is the preferred harvest method to regenerate aspen and provide age and size diversity.

Aspen regenerates in clearcut stands primarily by root suckering. Normally, suckering is prolific and dense sprout stands will persist until competition, diseases, and insects reduce the density. However, in some stands in the Rocky Mountain region, 90–100% of the sprouts die within 2 to 8 years after harvest. This extensive mortality results in regeneration failure and loss of cloned root systems (Crouch 1986).

The importance of shoot diseases of aspen suckers in regenerating stands is not well documented in Colorado. However, several studies indicate significant injury and damage to suckers occurs in Colorado (Crouch 1983, 1986; Hinds and Shepperd 1987). On Stoner Mesa in southwestern Colorado, Crouch (1983) found a 23% reduction of suckers in clearcut areas after 7 years. Forty percent of the remaining suckers were injured basally, and 57% were discolored internally or decayed. Crouch (1986) later reported continuing losses in the same areas. Some clearcuts had no live sprouts, while others had few sprouts, causing understocking or regeneration failure. Information on the causes of this sprout death is limited, but regeneration problems are usually attributed to browsing, high water tables, frost, and diseases (DeByle and Winokur 1985). The areas used in this study had no obvious high water tables or frost damage. A recent survey of these and other sprout stands in the region provided a few suggestions of vegetation differences be-

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tween stands with adequate or inadequate regeneration (Hildebrand and Jacobi 1990). Inadequately stocked stands had more yarrow (*Achillea lanuloss* Nutt.), dandelion (*Taraxacum officinale* Wiggers), strawberry (*Fragaria* sp.), and corn husk lily (*Veratrum tenuipetalum* Heller). Adequately stocked stands had more sweet cicley (*Osmorhiza* sp.), snowberry (*Symphoricarpos* sp.), and meadow rue (*Thalictrum* sp.). In a few sites where the slopes differed between adequate and poorly stocked stands, the poor stands were on level or low slope positions. However, there were too few samples of this condition to make any significant inferences. These vegetation differences may be indicators of sites with inadequate regeneration, but the data were collected after most sprouts had died so there could have been large differences in soil temperature and solarization that may influence vegetation composition.

Diseases of aspen sprouts have been studied in Colorado (Hinds and Shepperd 1987) and Minnesota, Wisconsin, and Ontario (Basham and Navratil 1975, Gross and Basham 1981, Perala 1984, Pollard 1971, Smith 1973, Stanosz and Patton 1984). The most common diseases and fungi associated with diseased sprouts were Armillaria root disease (*Armillaria* sp.), shoot blight [(*Venturia macularis*) (Fr.) E. Muller and Von Arx]; and cankers caused by *Dothiora polyspora* Shear and Davidson; *Cytospora chrysosperma* (Pers.) Fr.; *Pleurostromella* sp.; and *Sirodothis populina* (Thum.) Sutton and Funk. The objectives of this study were to (1) determine what fungi were associated with dying aspen sprouts in clearcut areas with greater than 90% mortality, and (2) determine the potential of these fungi as primary pathogens of aspen sprouts.

Study Sites and Methods

Four Colorado stands with aspen sprout dieback were visited, and dying sprout specimens were collected between May and September 1988. Carter Mountain, on state land near Kremmling and adjacent to the Arapaho-Roosevelt National Forest (T4N, R81W, sec. 23), was visited three times in 1988. Dying sprouts were collected on each visit. The second study area on the Pike National Forest near Jefferson (T7S, R76W, Sec. s4) was visited in June and August 1988. The third area was sampled in May 1988 on the Collbran District, Grand Mesa National Forest (T11S, R94W, sec. 4 & 9). The fourth area was visited in August 1988 and is located at the Fraser Experimental Forest (T2S, R76W, sec. 3 and T15S, T76W, sec. 34) near Fraser. A fifth study area at the Lakeside Ranger District, Apache-Sitgraves National Forest, near Lakeside, AZ, was sampled in August 1988. Five to 15 trees were sampled from each area with 50 to 160 isolations per site.

The Carter Mountain site was at the base of a slope. The entire hill (25.5 ha) was clearcut during the winter of 1983, but only the lower part, with <10% slope, had a high incidence of sprout mortality. This part of the sprout stand had a high incidence of shoot blight (*V. macularis*) in June 1987, and by August 1988, 75% of the stem on most sprouts had been killed by an unknown pathogen. The section of the stand with high disease incidence had scattered healthy plants, but most sprouts had died back to 0–20 cm from the ground. Sprouts were 0.75–1 m tall in the area with diseased sprouts, and 1.5–2 m tall on the area with mainly healthy sprouts. All randomly sampled trees in the diseased area had *C. chrysosperma* pycnidia on upper portions of stems. Several sprouts had *D. polyspora* fruiting bodies on small cankers.

The Jefferson site was a 2-ha aspen clearcut with <5% slope and 4-year-old sprouts. Most of the sprouts in the diseased portion (10%) of the stand were dead. Living sprouts exhibited cankers with orange margins and *C. chrysosperma* fruiting bodies.

The Collbran site was in a 4-ha stand with minimal slope that was clearcut in 1983 or 1984. Mortality was observed by the district silviculturist in 1986 and 1987. In July 1988 most sprouts were dead, indicating that most mortality occurred in 1986 and 1987. Chances of isolating pathogenic fungi from the sprouts were poor because the tissue had been killed for some time.

The Fraser site included three 20-m-diameter clearcuts where diseased trees were found on the shady side of the clearcuts. The sites were located at 2750 m elevation, in flat terrain, and were in previously mixed aspen and lodgepole pine stands that were 65 years old when cut in 1982. Sprouts in one area were damaged by browsing and snow breakage. The other two areas were relatively undamaged. Infections were not consistently associated with animal or snow damage.

The Lakeside, AZ, site was a wildlife clearcut, harvested in 1986. The site was in a level valley bottom. The site was fenced to limit domestic livestock, but the fence was broken in 1988 with considerable browsing as a result. Most sprouts had evidence of fungal cankers.

All sprouts sampled for isolation work were dying from the terminal with one or more cankers on the stem (fig. 1). Most sprouts showed symptoms and signs of Cytospora canker, i.e., black bark from the terminal of the plant down to an orange area where canker expansion was occurring along with fruiting structures exuding orange spore tendrils. The orange discolored bark graded into yellow and then green uninfected tissue.

Isolations from the leading edge of the cankered area on the main stem of diseased aspen were cultured on acidified potato dextrose agar (PDA). Peeling the outer bark with a sterilized knife reduced contamination more than an ethanol dip and flaming. Chips containing inner bark and xylem tissue from areas with orange discoloration most consistently yielded cultures of *C. chrysosperma*.

Pathogenicity tests were performed under controlled conditions utilizing the most commonly isolated fungi. Two isolates of *C. chrysosperma* obtained from dying aspen root sprouts at the Carter Mountain study site, and two isolates of *D. polyspora* obtained from dying aspen root sprouts at Fraser Experimental Forest, were used to inoculate eight 4-year-old aspen seedlings. The seedlings were grown in a greenhouse at 65 to 85°F in 19-liter pots in a mixture of 50% top soil, 25% sand,

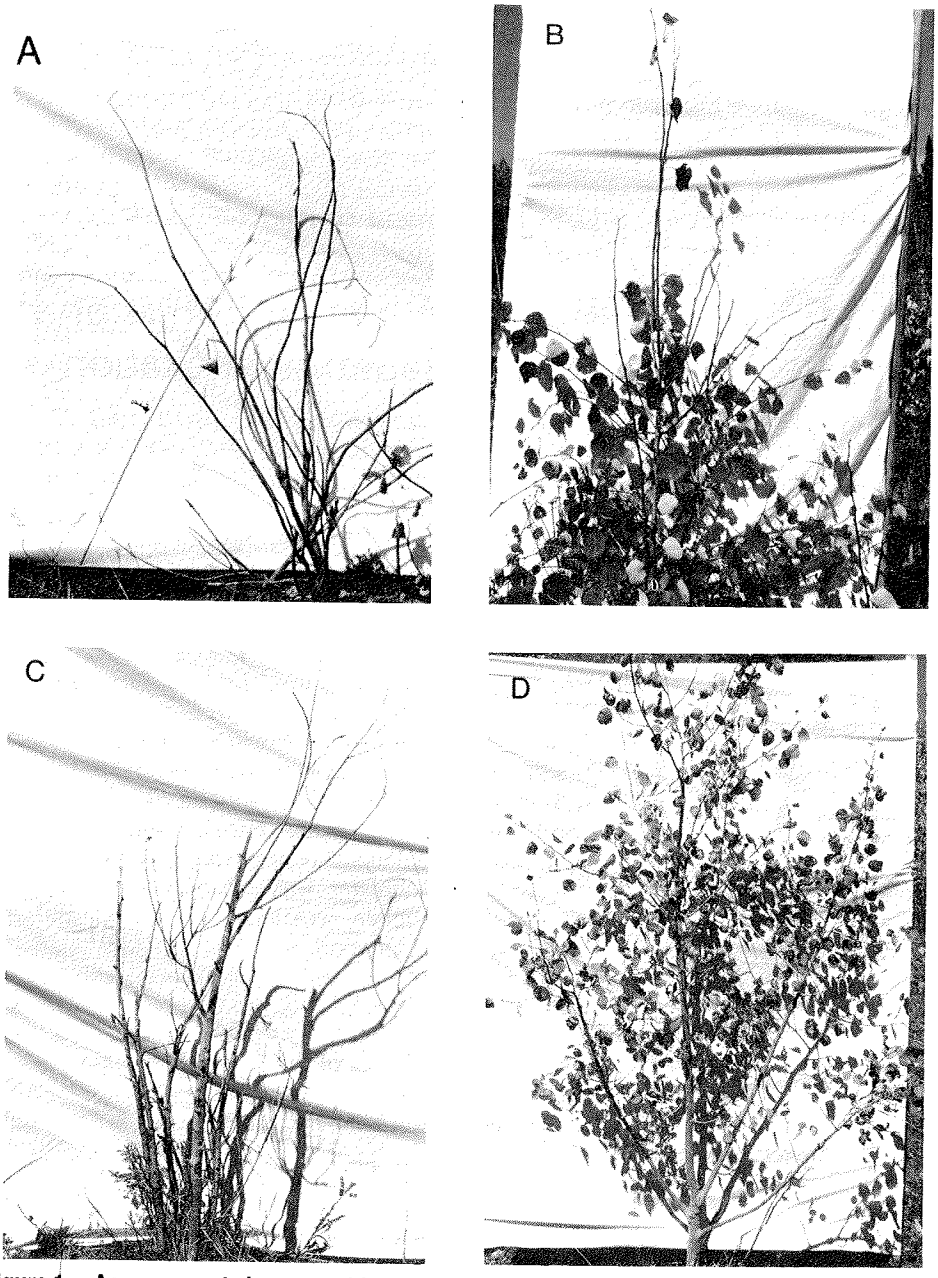

Figure 1.—Aspen sprouts in areas with regeneration failure and adequate stocking: on one site 3-year-old dead (A) and healthy (B) sprouts are ca. 35 and 120 cm tall, respectively; at another site 4-year-old dead (C) and healthy (D) sprouts are ca. 75 and 200 cm tall.

20% peat moss, and 5% composted wood chips and manure. The trees averaged 1.1 m tall and 1.5 cm in diameter. Three wounds were made on each of the eight trees by crushing the bark with a sterilized cold chisel, forming a 20-mm-long rectangular wound. The wounds were randomly inoculated with the four isolates giving a total of six inoculations per isolate. The isolates were grown on acid PDA at 23 °C with 8 hours of light per day for 7 days prior to inoculation. Agar plugs were placed in the wounds and parafilm was wrapped over the wounds. Inoculations took place March 30, 1989, and canker sizes were recorded and reisolations made 19 days later. Chips of bark and wood from canker margins were plated out on acid PDA.

Results and Discussion

Cytospora chrysosperma was isolated from 0–85% of the sprouts and 32% of the 909 chips from diseased sprouts (table 1). We experienced difficulty in obtaining pure cultures from the early collections; thus the occurrence of Cytospora isolations increased on subsequent samples. Several fungal contaminants out-competed *C. chrysosperma* colonies, but *C. chrysosperma* could be identified by waiting 4–6 weeks for pycnidia to form and reisolating from the spore tendrils. *D. polyspora* (Shear and Davidson 1940) was occasionally isolated from sprouts at Carter Mountain and Fraser Experimental Forest. No other pathogenic fungi were isolated.

Table 1.—Summary of isolations made from dying aspen sprouts.

Stand location	Collection date	Mortality noted	Sprouts sampled	Cysospora isolated	Dothiora isolated	Total isolations	Organisms		None
							Cytospora	Other[1]	
				----- percent -----					
Carter Mt, CO	5–20–88	1987	15	0	0	160	0	67	93
Carter Mt, CO	6–12–88	1987	12	75	0	165	71	94	0
Carter Mt, CO	8–12–88	1987	13	85	5	168	120	12	36
Jefferson, CO	6–15–88	1987	8	75	0	65	25	0	40
Jefferson, CO	8–04–88	1987	5	20	0	80	4	36	40
Collbran, CO	5–28–88	1986 + 87	11	0	0	55	0	9	46
Fraser, CO	8–27–88	1988	10	70	30	168	56	76	36
Lakeside, AZ	8–29–88	1987	4	75	30	48	19	29	0
Total			78			909	295	323	291

[1]*"Other" were considered contaminants, including bacteria; except that Dothiora polyspora was isolated 24 times at Fraser and once at Carter Mt.*

At least 80% of all sprouts collected had *C. chrysosperma* fruiting bodies on the stem portions that had been dead for 1 to 2 years. On some sprouts at the Fraser site, Cytospora was fruiting within 2 mm of the leading edge of the canker. Fruiting bodies of *D. polyspora* were found occasionally on the dead bark of sprouts from Carter Mountain and Fraser Experimental Forest. At Fraser, 7 out of 10 trees sampled had *D. polyspora* pycnidia close to canker margins. Dothiora cankers seemed to be restricted in size whereas the Cytospora cankers were expanding rapidly. No other pathogenic fungi were found on the stems. No evidence of root diseases, such as Armillaria, was found.

The pathogenicity tests conducted on the greenhouse-grown seedlings indicated Cytospora isolates were capable of causing infections, whereas Dothiora isolates did not induce cankers under the inoculation system used (table 2). One Cytospora isolate caused large cankers, whereas the second Cytospora isolate induced bark and cambium necrosis but little canker expansion. The similarity of symptoms of cankers on root sprouts and inoculated aspen seedlings, the presence of fruiting bodies at canker margins on sprouts in the field, and the pathogenicity of the Cytospora isolates indicate that Cytospora canker is most likely the major disease associated with the aspen sprout dieback observed in the study areas.

Neither isolate of *D. polyspora* caused cambium or bark necrosis, nor was the fungus isolated from the inoculated wounds on the greenhouse-grown seedlings. Since conditions and wound type that allow infection are not known for *D. polyspora*, we cannot conclude that it is not a pathogen. *D. polyspora* is probably a pathogen associated with some sprout mortality, but this conclusion awaits further investigation.

Table 2.—Vertical canker size on greenhouse-grown aspen seedlings inoculated with *Cytospora chrysosperma* and *Dothiora polyspora*.

Isolate (collection number)	Average canker size[1] (mm)	Wounds with visible bark necrosis
Cytospora (88–7)	25.3 a	6/6
Cytospora (88–5)	50.0 b	6/6
Dothiora (88–9)	21.5 a	1/6
Dothiora (88–8)	20.3 a	0/6

[1]*Average vertical canker size of 6 cankers, randomly located on 8 trees; means followed by similar letters are not significantly different as tested by analysis of variance and LSD ($P \leq 0.05$).*

The conditions that allowed Cytospora cankers to kill normally resistant trees in these regenerating clear cuts are unknown. Previous research indicates, however, that fluctuating soil moisture and defoliation stress may predispose aspen to rapid canker expansion by *C. chrysosperma* (Bloomberg 1962, Guyon 1990, Ramaley et al. 1987). Wounds are necessary for this pathogen to infect trees (Ramaley et al. 1987), and many wounds exist in nature from physical damages induced by ice, wind, animals, insects, and other diseases such as shoot blight. Careful monitoring of aspen regeneration and reporting of similar disease situations to the Rocky Mountain Forest and Range Experiment Station or Forest Pest Management staff, Rocky Mountain Region, will help prevent further losses and help us develop management options.

Continuing research and surveys by the USDA Forest Service, including both the Rocky Mountain Forest and Range Experiment Station and the Forest Pest Management, Rocky Mountain Region; USDA Soil Conservation Service; and Colorado State University, will help determine the stresses and site conditions which enhance sprout mortality and help predict the occurrence of disease-induced regeneration failure.

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